



Welcome to WGIN4 !

Contents:

Project Background	Page	1-2
Research Updates	Page	2-12
Events / News / Publications	Page	12-13

Defra Wheat Genetic Improvement Network (WGIN4): Improving the resilience of the wheat crop through genetics and targeted traits analysis

Section 1 Project Background

The UK government is committed to more sustainable agriculture, but this vision is facing an ever expanding range of environmental, energy and climate change challenges. **Wheat is grown on a larger area (1.8 m hectares) and is more valuable than any other arable crop in the UK.** The final estimate of the 2022 UK wheat harvest was 15.5 million tonnes. **The Wheat Genetic Improvement Network (WGIN) started in 2003, is funded by the Department for Environment, Food & Rural Affairs (defra).** The overall aim of WGIN is to generate pre-breeding material carrying novel traits for the UK breeding companies and to deliver accessible technologies and new knowledge, thereby ensuring the means are available to produce new, improved varieties that require lower inputs to produce the same or greater yields. To achieve WGIN's goals, an integrated scientific core was established which combines underpinning work on molecular markers, genetic and genomic research, together with novel trait identification. The programme is managed by a team including representatives of the key UK research groups and

breeders. They ensure the programme and its outputs are communicated to the wider scientific and end user communities, via a web site (www.wgin.org.uk), an annual stakeholders' meeting and peer reviewed publications.

The **4th phase of WGIN (WGIN4)** started in February 2018 and was initially funded for 5 years but has since received an additional 1 year funded extension to complete the research goals affected by the Covid-19 pandemic. WGIN4 is entitled '**Improving the resilience of the wheat crop through genetics and targeted traits analysis**'. This project consisted of four work packages (WPs) (**Figure 1**). WP1 focusses on further enhancing the networking and communication activities. **Two new activities** are included in WP1. Firstly, via the newly formed project management group (PMG) and the research advisory group (RAG), the WGIN project has been aligned to the other three defra funded GINs, namely PCGIN for pulse crops, OREGIN for oilseed rape and VeGIN for field and leafy vegetables (<http://www.wgin.org.uk/about/GINs.php>). Secondly, AHDB has become involved to a far greater extent in disseminating the GINs activities and our key research findings to UK based farmers and crop advisors. This is being done through AHDB's strategic farm and monitor farm events programme. AHDB is also helping to promote the annual GINs stakeholder events more widely.

Within WGIN 4 the three inter-connected research work packages (WP2, WP3 and WP4) (**Figure 1**) remain the same as in WGIN3. They are focussed on exploring a range of previous and newly nominated high priority traits for the

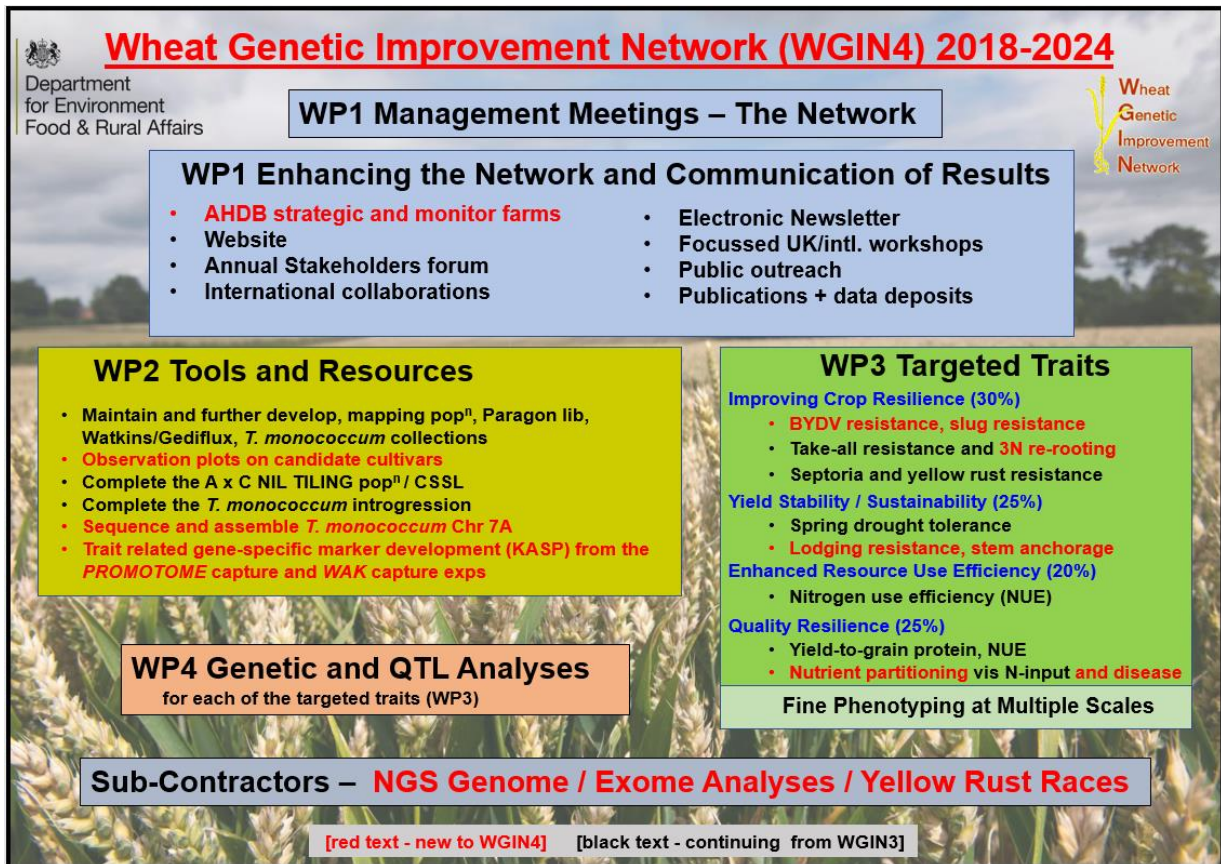


Figure 1: The organisational schematic of WGIN4. All red text highlights new additions compared to WGIN3

UK wheat crop, followed by detailed genetic and quantitative trait loci (QTL) analyses (WP3 and WP4), maintaining and developing new genetic resources for the UK research community (WP2), and testing new tools based on next generation sequencing technologies for their applicability to wheat research (WP4). In WGIN4, 70% of the research effort is focussed on traits analyses. The split of this research effort between the four overarching traits is resilience (30%), yield stability/ sustainability (25%), quality resilience (25%) and resource efficiency (20%).

The core funded partners in WGIN4 are the **John Innes Centre (JIC)** and **Rothamsted Research (RRes)**.

In this **May 2023 newsletter** we are providing an update on some of the research activities and results that have been achieved over the past two years.

Section 2 Research Updates

WGIN4 at the JIC

• Yield Stability & Sustainability Traits

After the extreme weather of last summer, the graphs of temperature and rainfall at the JIC Experimental Station in Bawburgh, Norfolk (**Figure 2**) are even more dramatic than previously. These data indicate how volatile and unpredictable the weather conditions have become due to climate change, with increasing temperatures and erratic rainfall, making growing conditions for wheat in the UK even more challenging for both farmers and wheat researchers alike.

The 2019-2020 and 2021-2022 growing seasons were generally hotter and drier than the 2020-2021 season. It's very difficult to imagine what the summer of 2023 will bring though current predictions suggest that conditions might be quite cool and damp until early summer... April has certainly been wet and cool! These fluctuations in temperature and rainfall will inevitably be reflected in yield variation.

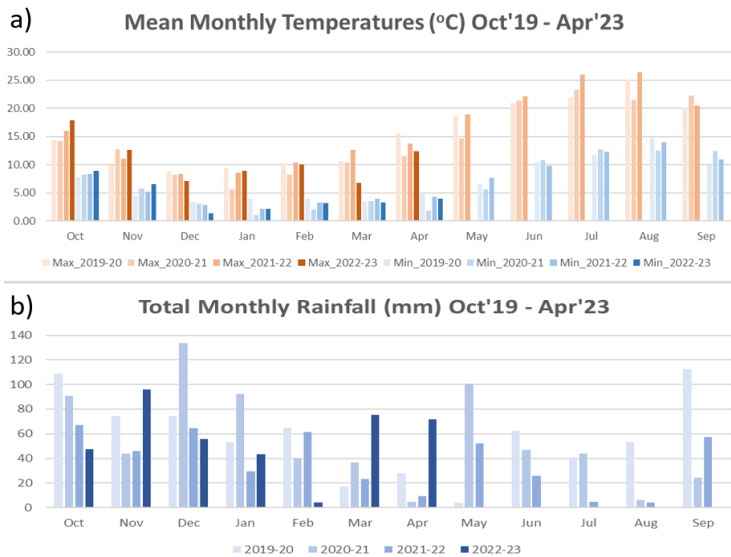


Figure 2: a. Graph shows the mean monthly minimum and maximum temperatures (°C) over the 2019-2020, 2020-2021 and 2021-2022 growing seasons, and part of the 2022-2023 season. b. Graph shows total monthly rainfall (mm) over the 2019-20, 2020-21 and 2021-22 growing seasons, and part of the 2022-23 season

At JIC we are continuing our work to produce wheat with greater resilience to the variable weather conditions, to maintain total yield and grain quality regardless of the environmental conditions.

As shown in **Figure 2b** rainfall has been very variable over the last 3 to 4 seasons and East Anglia is still in a drought situation, despite the heavy rain in March and April 2023. Put together with the rising spring and summer temperatures then plants increasingly suffer from both drought and heat stress. In addition, extreme weather, such as heavy rain and strong winds, can cause physical damage to the plants through lodging. All these factors have a serious negative impact on yield. We are attempting to improve drought tolerance and resilience to reduced rainfall as well as studying how the wheat plant can physically withstand extreme weather.

- **Dissecting UK drought tolerance in Paragon x Garcia**

We have previously extensively reported on our drought trials with a Recombinant Inbred Line (RIL) population from a Paragon x Garcia (P x G) cross. Our analyses shows that the most important QTL for yield is found on chromosome 2B in Garcia. Previous data suggested that this Garcia allele can increase grain size *and* grain number helping plants survive both spring and summer drought. We have now confirmed this result in a full yield trial in which lines carrying the Garcia version of

this gene increases yield compared to lines which carry the Paragon version (**Figure 3**).

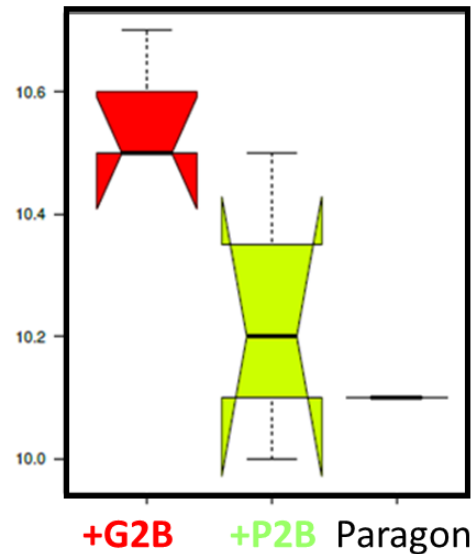


Figure 3: Graph shows the spread and means of Grain Yield (GY) in t/ha of NILs carrying the Garcia (red / +G2B) or Paragon (green / +P2B) 2B alleles, plus the Paragon control.

In addition, we tested selected lines from the P x G RIL population for root anchorage in a +/- irrigation trial in 2021 and some RILs showed particularly good anchorage in dry conditions. This suggests the wheat plants may have strong or deep roots which can help the plants explore further and find water in drought conditions. In summer 2022 we tested anchorage on all the P x G lines used in the original drought trials, as well as Paragon and Garcia. Garcia was shown to have stronger roots than Paragon, while strong anchorage was observed in some of the RILs.

- **Lodging and Anchorage**

We are now concentrating on the effect on crop yield and quality due to lodging. There are many non-biotic factors that contribute to lodging occurring but here we are mainly looking at weather or irrigation-induced lodging. There are likely to be many factors that could affect how a plant responds to conditions which induce lodging; time of ear emergence, plant height, stem strength/ stiffness/ thickness/ composition, ear weight, ear/stem number, anchorage and root depth and probably more! We have measured or assessed a number of these phenotypes in the field, from the air and post-harvest, and have used these data to locate chromosomal regions which might contribute to lodging resistance.

From our 'Drought, Anchorage and Lodging Panel' (DALP), seasons 2019-2020 and 2020-2021 (+/- irrigation) we identified some varieties of particular interest and we have followed a few of these up using **Recombinant Inbred Lines** (RILs) from these varieties crossed to Paragon; Paragon x Joss Cambier (P x JC), Paragon x Watkins110 (P x W110) in 2020-21, Paragon x Starke (P x St) and P x G in 2021-22 (see below).

As our research focus had shifted more towards lodging and anchorage, we set up a five-rep trial, without irrigation, with a subset of varieties from DALP (mainly elite varieties and the Rothamsted panel), PxJC and PxW110, (2021-2022), to analyse this, known as the **Midi-Lodging Trial**. Flowering time (DTEM) and height (HT) were recorded as normal. Plot strength was determined using a movable device for measuring stem strength (**Figure 4**). This is a modification from the original "PushOMeter" (see Newsletter 2021).



Figure 4: Push-O-Meter v2; device for measuring plot strength.

Ear counts (= stem count) were made for 1m of plants/plot. Stem strength was calculated from the plot strength and ear count values. Values for yield (YLD), specific weight (SW) and straw weight were collected at harvest, and TGWT measured as normal.

We were hopeful that this trial would give us clear results... Unfortunately, due to the extremely hot and dry summer weather in 2022, the majority of lines were not tall, so less susceptible to lodging. In addition, the lack of rainfall and strong winds meant that no lodging was observed in any lines, except W110 and its RILs (**Figure 5a**). Interestingly, despite being very tall (>1.6 m), most of the lodged W110 and RILs were able to recover to an extent. We believe this is due to very thick but flexible stems (**Figure 5b**)

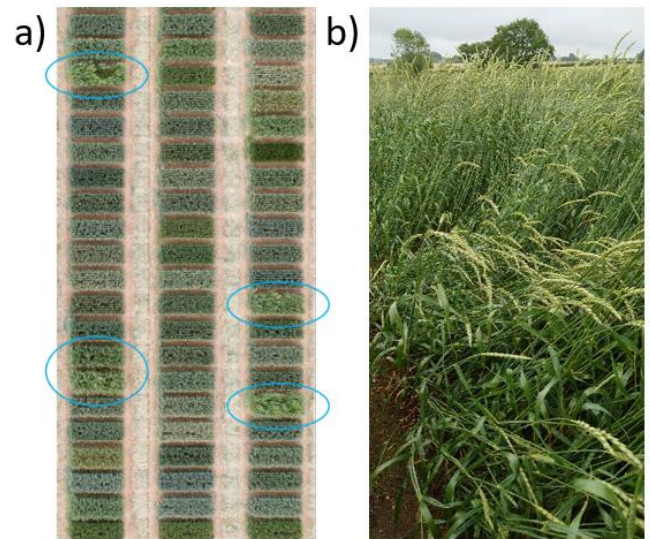


Figure 5: a) Section of the 2021-22 Midi-Lodging Trial. Plots showing evidence of lodging are ringed in blue. b) W110 RIL immediately after severe weather; most of these plants became more upright later in the season.

The Paragon x Starke (P x St) and Paragon x Garcia (P x G) RILs were drilled as 1m plots in autumn 2021, without irrigation, the P x St were duplicate plots. Both populations were scored for DTEM and Ht, plus anchorage, stem number and anchorage/stem. Anchorage measurements were made using a modified 'Pull-O-Meter' (**Figure 6**).

All quantitative data was analysed by QTL mapping. No QTL were identified for anchorage and anchorage/stem for P x G but, as mentioned above Garcia has stronger roots than Paragon, and variation in root strength was observed in the P x G RILs.



Figure 6: "Pull-O-Meter" v2; device for measuring root anchorage

The results for the P x St RILs were much clearer! **Starke** is a Swedish variety and is tall and late flowering. The

variety was used in both the DALP and Midi-Lodging trials. Starke showed weak plot/stem strength in both trials and high anchorage in DALP. Lodging did not occur in either trial, despite Starke being tall and having weak stems.

This quantitative data from the trial gave a strong QTL on Chr 5A for flowering (DTEM), with Starke as the increasing allele (**Figure 7a**). Initially this was thought to be *Vrn1A* but we now believe we have identified a different candidate, a known flowering time gene in Arabidopsis involved in the circadian clock. This could be relevant for wheat grown in an environment of considerable differences in day length over the course of a year such as in Sweden. A second QTL on Chr 7B was also identified.

QTL mapping using the anchorage data also produced a QTL on Chr 5A, which is closely linked, but distinct from the flowering QTL (**Figure 7b**) and we have a plausible candidate for this QTL as well.

Due to the high anchorage value of RILs carrying the Starke allele, roots from selected RILs, plus Paragon and Starke were dug up, washed and then examined. **Figure 8** clearly shows that Starke roots are much more substantial than those of Paragon, and the root phenotype was segregating in those RILs examined.

We therefore believe that the strong root phenotype of Starke is a major factor in preventing lodging due to strong anchorage. In addition, the weak stem strength phenotype,

even though it has a moderately thick stem wall, likely gives flexibility to the stems and helps to withstand lodging events. It is also possible that Starke roots could help the plant withstand drought conditions, despite it being bred to grow in Sweden. Sweden is not hot compared to S. Europe so 'drought' may not be the same phenomenon in both areas? If Swedish winter conditions are cold (sub-zero) and precipitation is low, then water availability will be reduced and it is technically a 'drought'. We have some evidence (data not shown) that the roots of young Starke plants are growing down into the soil as quickly as possible in autumn. An additional benefit of this could be the added stability of deep roots during extreme winter weather.



Figure 8: Typical roots from three Paragon and three Starke plants showing the more robust phenotype of Starke roots.

Many thanks especially to **Hannah Carthy** for almost all the field and post-harvest phenotyping, plus **Perrine Lesniarek**, also **Phil Robinson** and **Rich Samworth** for the drone imaging in 2022.

Our final trial within WGIN4 is an **irrigation trial** using 100 of the P x St RILs, plus Starke and Paragon controls, to **explore drought, anchorage, and lodging**. The trial consists of two randomised reps that will be irrigated (IR) and two randomised reps that will not be irrigated (NI). Treatments will be applied to maximise lodging pressure (high nitrogen and no PGR) as well as early drilling (06/10/2022) with a high seed rate. The field chosen for the trial has fine free-draining sandy soil which should make the extraction and examination of roots easier, as well as accentuating the difference between the IR and NI reps. We are interested to determine if the unusual root phenotype in Starke affects yield under differential irrigation. Is there a yield penalty/advantage to having 'Starke' roots in drought conditions? We also want to determine how much drought/irrigation affects anchorage and lodging. We will perform our usual trial phenotyping: Flowering time (DTEM), Height, Yield, Specific Weight, TGWT and regular drone observation for lodging and the effect of irrigation. In addition, anchorage measurements will be taken at anthesis, and stem count performed. There will be a comparison of above/below ground biomass between the

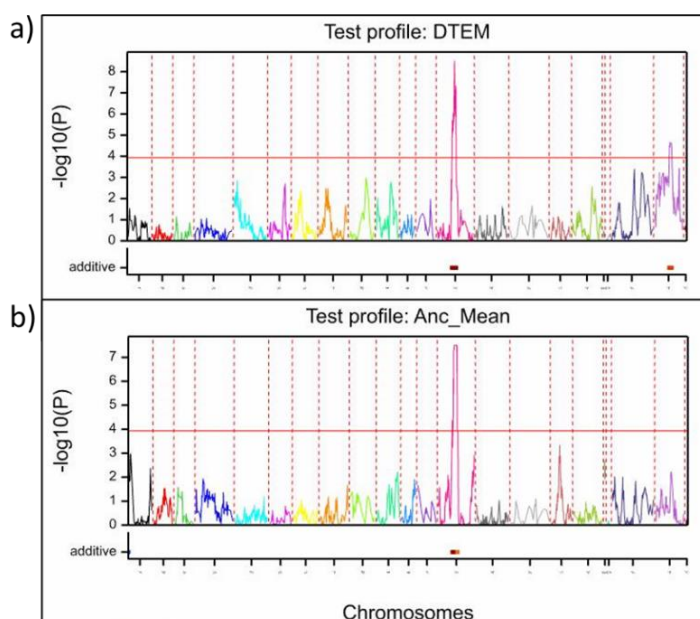


Figure 7: **a)** Figure shows the position of the flowering time (DTEM) QTLs on Chr 5A and Chr 7B. **b)** Figure shows the position of the anchorage QTL on Chr 5A. The Chr 5A positions look very similar on the graphs, but they have different QTL peak markers nearly 20 Mb apart.

root phenotypes. If possible then additional phenotyping will be performed; digging up roots and measuring root angles and root depth, stem strength measurement with the Push-O-Meter, stem width and stem wall measurements. The quantitative data will be used for QTL mapping, where we hope to refine the QTLs already identified and possibly detect new QTLs. Qualitative data will also be generated through a detailed examination of the roots.

The QTL for anchorage from Starke has already been nominated for the Breeders Tool Kit as we believe this is likely to be an important trait towards breeding lodging resistant wheat and hopefully more drought tolerant wheat able to maintain or even increase yield under drought conditions.

- **Resistance to Slug Damage**

We have previously identified a Watkins landrace with high degree of resistance to slug damage; Watkins788 (W788). We have now bulked sufficient seed for farm-based trials which was distributed to participating farms in autumn 2022 (**Figure 9**). This is a collaboration with the BOFIN network coordinated by Tom Allen-Stevens. At the recent WGIN Stakeholders meeting (6th February 2023 @ JIC) Tom reported their initial results (recording available on the WGIN homepage). Unfortunately the dry autumn of 2022 meant there were few slugs present in the plots so it was difficult to assess the performance of W788. The farmers will be repeating the trial from autumn 2023. This work has led to our involvement in projects which take the initial W788 results to the next stage with additional funding from the Environment Agency and the Defra Farming Innovation Programme.



Figure 9: Bulking of Watkins788 at Church Farm, Bawburgh, Norfolk

For further information on this aspect of the WGIN project contact **Clare Lister** (clare.lister@jic.ac.uk) or **Simon Griffiths** (simon.griffiths@jic.ac.uk) at the **John Innes Centre**.

New Drone Capability at Rothamsted (RRes)

During 2021 a new drone and sensing technology was used over the WGIN Diversity field experiment for the first time. The drone (**Figure 10**) carries a co-aligned VNIR/SWIR and lidar sensor system; the VNIR sensor produces images with 270 bands covering visible to near infrared wavelengths (400-1000nm, 2.2nm/band), the SWIR 267 bands covering shortwave infrared (900-2500nm, 6nm/band). The images are 640 pixels by just 1 pixel. This is known as a 'push-broom' system, and to obtain a two-dimensional image multiple 1 pixel wide images have to be stitched together. For accurate stitching the sensor needs to capture precise data on its orientation and geo-location when each image was taken. The orientation is measured by a high precision onboard **inertial measurement unit** (IMU), and the location is recorded by GPS. During the flight, GPS data is also collected by a base station on the ground, and when the data is processed after the flight, the data from the base station is used to correct the drone GPS data, making it much more accurate – known as **post processing kinematics** (PPK). This technology does not require ground control points for geo-referencing the images. Finally, to produce accurate two-dimensional images it is necessary to know how far the sensor was from the subject, and for this the data from the on-board lidar is used. The 16 channel lidar builds a very accurate three-dimensional image of the ground during the flight, measuring 300 000 points/second, and from these data, every pixel from the hyperspectral sensors is accurately geo-located in three dimensions. The hyperspectral data is calibrated using reference targets of known reflectance on the ground.



Figure 10: Hyperspectral and lidar capable drone, deployed at Rothamsted since 2021

The result is a high accuracy hyperspectral geo-located image (**Figure 11**) as well as a 3D point cloud. In-house software, written by a previous PhD student, has been adapted so that a shape file of the plot layout can be overlaid, and plot data extracted into a spreadsheet. So far, plot mean data is being calculated, but in the future, it is expected that intra-plot variability will be used to explain plot heterogeneity, and possibly biotic and abiotic stresses. From the plot data various growth and stress indices can be calculated, and the WGIN trial with the three levels of nitrogen fertiliser applied, 20 diverse varieties and two levels of agrochemical inputs to control pests and diseases, is an ideal experiment to test the technology and determine what trait data can be extracted from the imagery.

For further information on this aspect of the WGIN project contact Andrew Riche (Andrew.Riche@rothamsted.ac.uk) or Malcolm Hawkesford (Malcolm.Hawkesford@rothamsted.ac.uk) at Rothamsted.

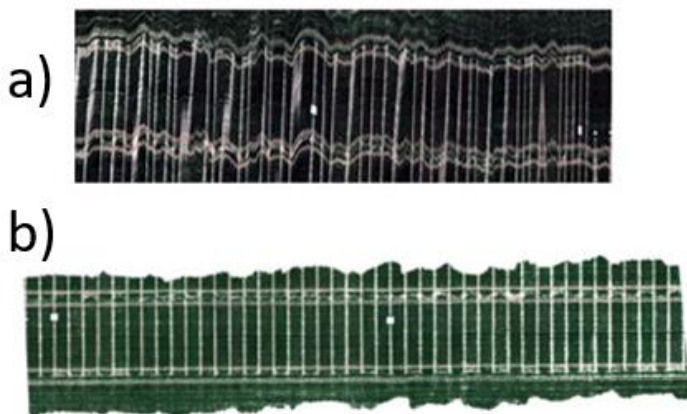


Figure 11: Examples of (a) uncorrected data as captured during the flight, and (b) after post-processing, using the GPS and lidar data to produce a geo-located image, in this example of a line of wheat plots in the WGIN Diversity trial in 2021.

Testing take-all resistance in the 3N introgression from *Aegilops uniaristata* into Chinese Spring (RRes)

Aegilops uniaristata is a **wild wheat relative** with reported **higher aluminum tolerance** than wheat (Berzonsky and Kimber 1986). Introgression lines in which the 3N chromosome from *A. uniaristata* replaces the homoeologous wheat chromosomes, 3A, 3B or 3D of Chinese Spring wheat have been produced and they also show the increased aluminum tolerance (Miller et al 1997).

The aluminium tolerance has been shown to be related to prolific root growth. To test if the prolific growth phenotype by the 3N introgression also provides tolerance to root diseases like take-all disease, four introgression lines provided by Claire Lister from the John Innes Centre, and the corresponding Chinese spring wild type line, were tested for take-all tolerance. Due to the difficulty of multiplying the seeds of these introgression lines, initially the intended field experiment plans with third wheats under high take-all disease pressure were not possible. Instead, we performed an experiment in semi-field conditions using large pots in a greenhouse. Pots containing take-all inoculum and the introgression lines were prepared in November 2020 and the plants were assessed in summer 2021 for both aboveground and belowground phenotypes. The aboveground phenotypes measured consisted of plant height, flag leaf length, ear length, spad meter measurements of chlorophyll content at growth stages GS45-47 (booting), GS59 (ear emergence) and GS61 (start of flowering), time from booting to anthesis, tiller number and ear number. Grain measurements were not possible because rodents managed to enter the greenhouse and eat the grain of most plants. No significant differences were found for any of the aboveground traits between infected and uninfected plants as shown in **Figures 12** and **13**.

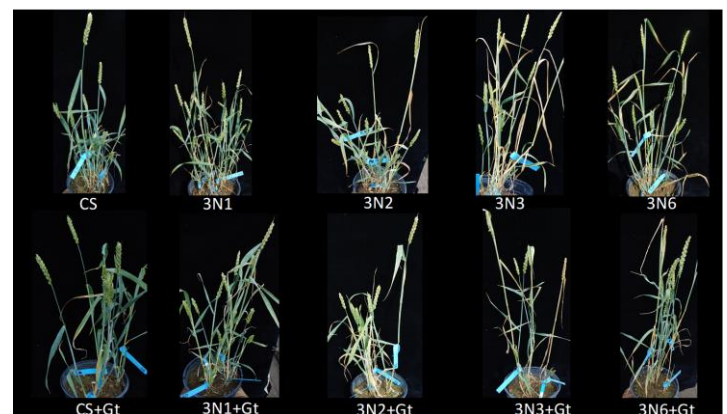


Figure 12: Pots of 3N introgression lines and Chinese Spring uninfected (top) or infected with take-all (bottom, Gt = *Gaemannomyces tritici*, the fungus causing take-all disease).

Below ground measurements consisted of root number, root length, root dried biomass and take-all index. All the lines were severely affected by the root damage caused by the take-all infection, and only one of the introgression lines (3N3) showed significantly lower take-all index levels compared to the Chinese Spring control line, but this difference was subtle and no significant difference was observed for the number of roots per plant or the average root biomass per plant (**Figures 14** and **15**).

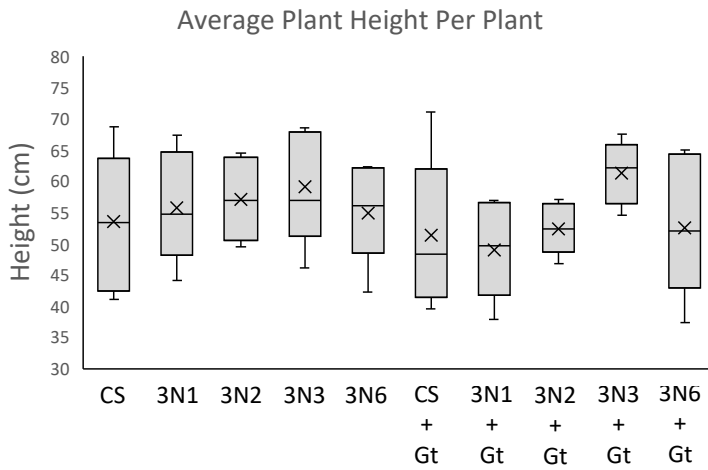


Figure 13: Height values, measured from stem base to the top of the ear of the main tillers for the different introgression lines and for the Chinese Spring wild type line in absence and presence of take-all. No significant differences were observed by take-all infection for any line. The bars represent standard error.

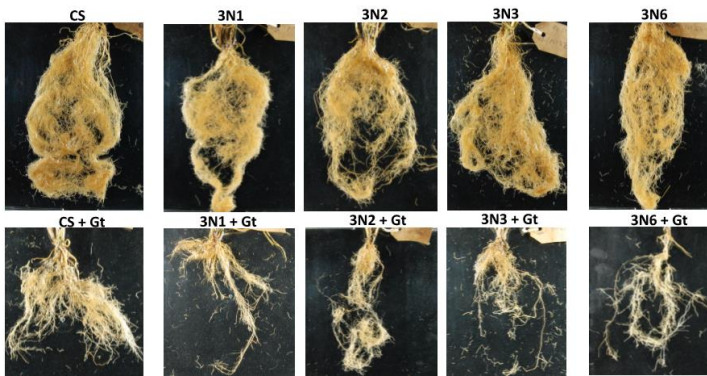


Figure 14: Root system of the different lines in absence (top) or presence (bottom) of take-all disease.

These results suggest that the **3N chromosome introgression of *A. unaristata* into wheat does not provide take-all tolerance**. This may be due to the fact that take-all affects the upper root area closer to the root crown and not the area of root elongation, so the prolific root growth of these lines would not provide protection to the root damage caused by take-all disease.

For more information, please contact **Gail Canning** (Gail.Canning@rothamsted.ac.uk) or message us on Twitter (@WheatGIN).

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- Miller TE, Iqbal N, Reader SM, Mahmood A, Cant KA and King IP. 1997. A cytogenetic approach to the improvement of aluminium tolerance in wheat. *New Phytologist* 137: 93-98.

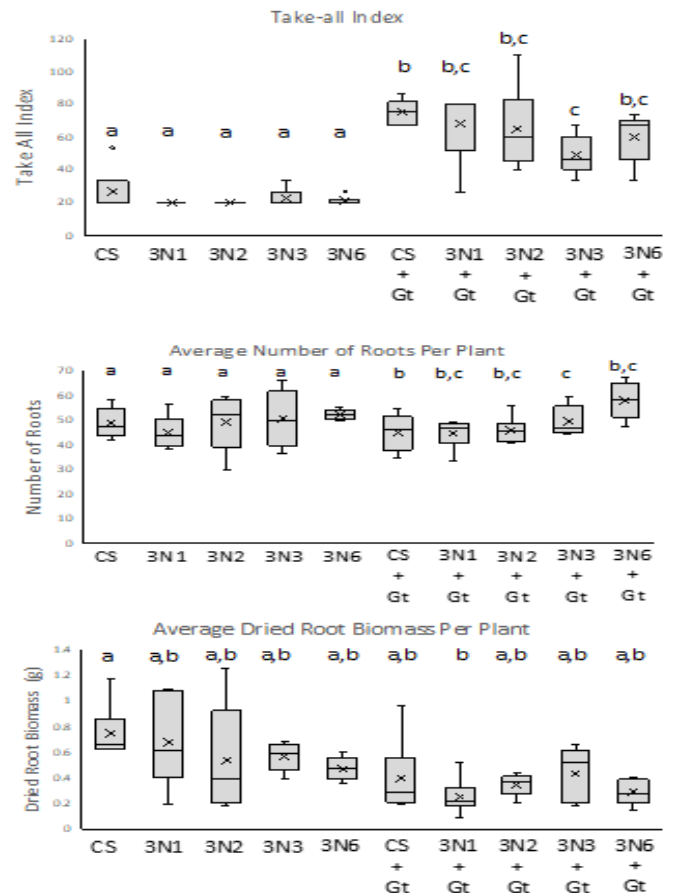


Figure 15: Belowground measurements for introgression lines and Chinese spring wild type in absence and presence of take-all. The bars represent standard error. The letters on top of the bars indicate the different significance groups.

Barley yellow dwarf virus (BYDV) and considerations for future disease control (RRes)

The English grain aphid (*Sitobion avenae*; **Figure 16**) and bird cherry-oat aphid (*Rhopalosiphum padi*) endure as a cause for concern to UK wheat growers. In addition to direct feeding damage, secretion of sucrose rich honeydew can promote saprophytic fungi, indirectly reducing photosynthetic ability and yields of an affected crop. Of greater impact, aphids also contribute to significant wheat yield losses through the transmission of viruses. **Barley yellow dwarf virus (BYDV)** is the most important wheat-affecting virus with discrete strains widely spread throughout the UK by *S. avenae* and *R. padi*. BYDV can cause yield losses ranging from 5-80% with a reported average of 30% (Aradottir Crespo-Herrera, 2021; DOI: 10.1016/j.cois.2021.01.002). Indistinct BYDV-associated visual disease symptoms, if apparent whatsoever, render this a difficult pathogen to effectively monitor and control.

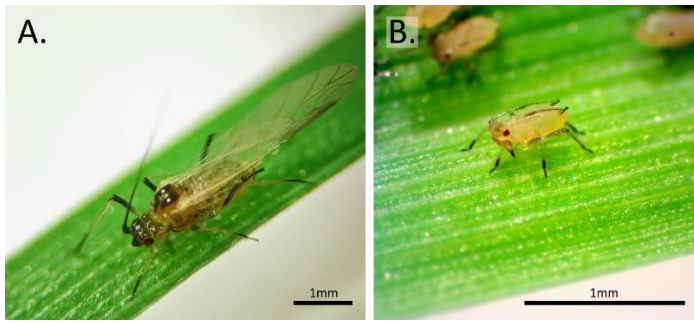


Figure 16: (A.) Winged *Sitobion avenae* adult and (B.) wingless offspring 'nymph' feeding on wheat leaves.

Pesticides have historically formed a principal line of defence against aphids and the diseases they transmit. Changes in pesticide regulations and potentially reduced mid/long-term effectiveness of current insecticide classes, however, have fuelled ongoing shifts towards alternative methods for disease and integrated pest management.

According to UK arable pesticide usage reports, aphids remain the foremost reason stated for why insecticides are applied to wheat, rising slightly from a markedly high 92% a decade ago in 2012 to a consistent 95% after 2016 (Various Garthwaite et al., 2012-2020; reports 250, 263, 271, 284 and 295; <https://pusstats.fera.co.uk/published-reports>). Several interesting trends in wheat insecticide use are emerging from these biennial reports, many of which may have significant impacts for future aphid and/or BYDV control. Overall use of insecticides has notably reduced in recent years. However, the diversity of insecticides available is increasingly limited due to both identified mode(s)-of action and what is permissible for use as new regulations are imposed. In 2020, the pyrethroid Lambda-cyhalothrin accounted for 82% of common spray formulations, another pyrethroid (Esfenvalerate) made up 11% with the remaining consisting of several additional pyrethroids. Resistance to pyrethroid-based insecticides is already established in clonal populations of *S. avenae* with moderate levels of resistance distributed across the UK. Alarmingly, evidence is emerging that *R. padi* may also be exhibiting resistance (Lael et al., 2020; DOI: 10.1564/v31_feb_02). The emergence of new insecticide-resistant aphid populations, or wider spread of those already identified, may pose significant challenges for future aphid/BYDV control.

• New BYDV strain diagnostic assays

Recent work at Rothamsted Research has explored UK-wide BYDV strain variation using aphid samples collected from the nationwide insect monitoring survey (<https://www.rothamsted.ac.uk/insect-survey>). **Partial BYDV**

genome sequencing from these samples has helped to identify strain-specific markers and suggested the presence of BYDV strains not previously identified in the UK. Shifts in sub-strain variation and the presence of undetected BYDV may have implications for effective BYDV disease management, especially as new BYDV-resistant wheat cultivars are deployed with an associated increase in selection pressure for resistance-breaking BYDV variants. A tangible output from this work has been the use of new BYDV sequencing data to develop refined UK-specific diagnostic assays. Based on PCR-based KASP genotyping (**Figure 17**), these new assays are highly sensitive and more readily accessible to industry than alternative molecular approaches. They can also be rapidly adjusted for specific BYDV variants of interest.

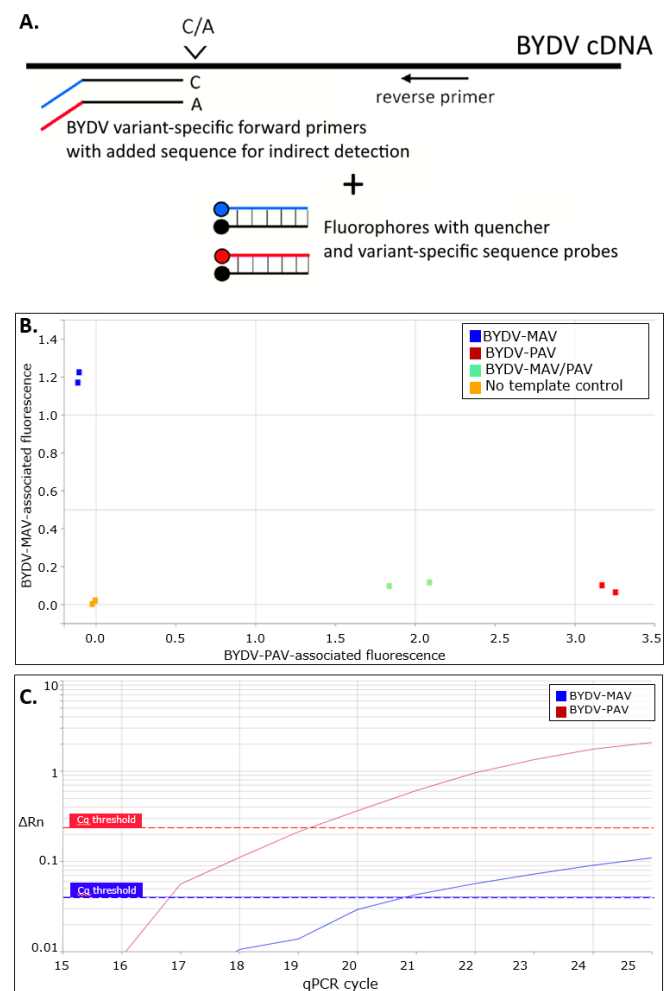


Figure 17: **A**, Components of new barley yellow dwarf virus (BYDV) variant-specific assays designed around accessible genotyping chemistry. Complementary DNA generated from reverse transcription of target samples with random hexamers is processed by PCR with variant-specific primers and fluorescence cassettes for indirect BYDV detection; **B**, Representative example of BYDV assay endpoint reading outputs with replicate samples containing BYDV strains MAV and PAV, and samples of a plant with mixed BYDV-MAV/PAV infection; **C**, Realtime semi-quantitative data of one mixed BYDV-MAV/PAV sample. Fluorescence data collected in addition to endpoint readings can be explored further to investigate semi-quantitative presence of discrete targets of interest.

• Further exploring resistance in *Triticum monococcum* to aphids

After extensive phenotyping for genetic sources of aphid resistance, *Triticum monococcum* lines showing resistance to both *R. padi* and *S. avenae* have been identified and used to develop mapping populations. The most noteworthy of these lines (MDR049) is being taken forwards for quantitative trait locus (QTL) mapping. This source of resistance has already been demonstrated as field viable with both pre-alighting and post-alighting resistance mode(s)-of-action (Simon et al., 2021; DOI: 10.1038/s41598-021-92883-9). Robust phenotyping methods investigating aphid nymph development have been developed and shown to generate data considered viable for QTL mapping. Complementing this work, the highly valuable WGIN promote capture data resource (Hammond-Kosack, M. et al., 2021; DOI: 10.1111/pbi.13672) has enabled the design of MDR049-specific KASP markers distributed across the genome. These validated markers will now form the basis for mapping post-alighting aphid resistance and assist with parallel work introgressing this valuable trait into commercial wheat breeding material.

We are keen to continue to actively engage with wheat growers and breeders who are happy for us to perform aphid and BYDV sampling in their fields. This would not affect wheat production in any way, we only need small samples from a few plants per field. Clonal aphid cultures have also been established for both *R. padi* and *S. avenae* with each routinely validated as vectoring BYDV strains PAV and MAV, respectively. This has enabled refined BYDV resistance screening to be performed.

For further information on this and any other enquiries related to ongoing aphid and BYDV research at RRes, please contact **Lawrence Bramham** Lawrence.Bramham@rothamsted.ac.uk

Update on Introgression of *Triticum monococcum* Into Hexaploid Wheat (RRes)

As reported in the 2021 Newsletter, the successful approach to introgression of *Triticum monococcum* (diploid, AA genome) into hexaploid *Triticum aestivum* wheat employed two tetraploid *Triticum durum* wheat cultivars, Kronos and Hoh501, as bridging species. **Table 1** shows the different stages of this crossing strategy as a reminder.

Table 1: The different stages of *Triticum monococcum* introgression into hexaploid wheat. Tdur= *Triticum durum*, Tm= *Triticum monococcum*, Taes=*Triticum aestivum*, F1C=F1 complex, BC=back cross

Stage	Crosses (♀ x ♂)	ploidy
Stage 1	Tdur x Tm = F1_hybrids	triploid
Stage 2	F1_hybrids x Taes = F1C	pentaploid
Stage 3	F1C x Taes = F1C_BC1	hexaploid(ish)
Stage 4	F1C_BC1 x Taes = F1C_BC2	hexaploid(ish)
Stage 5	F1C_BC2 x Taes = F1C_BC3	hexaploid

Introgression of the *Triticum monococcum* MDR031 (Take-all resistance) had reached the BC3 stage (stage 5) while aphid and Septoria resistance introgression (using MDR049 and MDR308, respectively) had completed BC2. After discussion with the UK wheat breeders at the WGIN4 Management Meetings and the fact that every round of backcrossing with Paragon reduces the amount of *T. monococcum* introgressed sequences further, we decided to halt the backcrossing, and instead self the current backcrossed lines in several rounds of single seed descent (SSD) to obtain as many near homozygous lines as possible. Further rounds of backcrossing may be restarted if the desired resistance is observed in any of the SSD lines to facilitate cloning of the resistance/trait genes. All lines underwent three rounds of ear bagged SSD (completed by late Spring 2022) followed by one round of grain multiplication under glasshouse conditions (ear harvest finished November 2022, grain cleaning, counting and weighing in January 2023). In total, **1022 individual lines** were multiplied in the glasshouse. For seed multiplication, three grains were grown for each line in one 2 litre, 5 in² pot in the glasshouse. All pots were fully bagged prior to anthesis to avoid any possible cross-pollination between lines. When anthesis had finished for all lines/ pots, bags were removed to avoid accumulation of potential pests and diseases (**Figure 18**).

Leaf discs were harvested from each line and genomic DNA extracted. We are now awaiting the commercial release (apparently imminent) of the next generation breeders' array, developed by Bristol University (Gary Barker and colleagues), to genotype all lines. This new array, called **TaNG**, has a considerably higher number of SNP markers than the 35K Breeders' array, and also a markedly improved distribution of markers between the A, B and D genomes. Together with the completed genomic

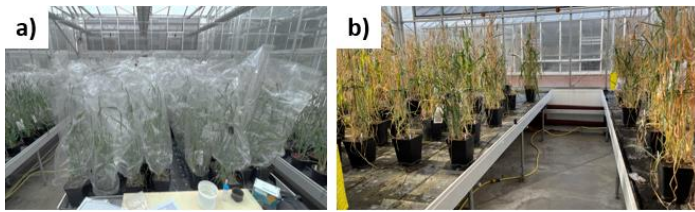
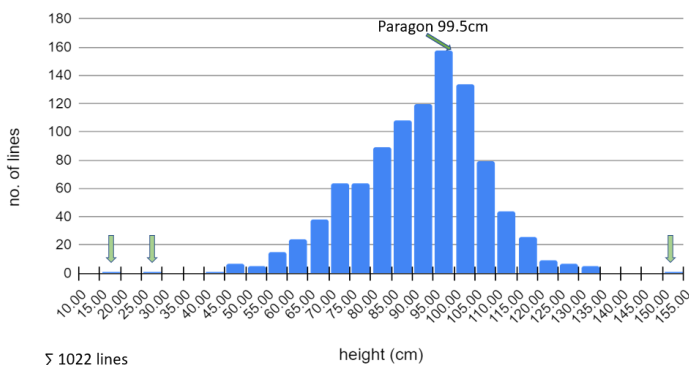


Figure 18: Seed multiplication in the glasshouse. In total 3 compartments were required to accommodate all 1029 lines (1022 introgressed, 7 Paragon controls) **a)** bagged lines awaiting leaf sampling for genomic DNA extraction **b)** unbagged lines awaiting photography, height measurements and ear harvest

sequencing of Paragon, Kronos and several *Triticum monococcum* accessions this will allow us to pinpoint the introgression events in our lines with high precision.

Figure 19 shows the height distribution of the introgressed lines, indicating the wide range of plant heights observed including three extremes at 15cm, 29cm (dwarfs) and 154cm. *Triticum monococcum* plants are notoriously tall, so any lines significantly taller than Paragon are expected to have the *T.mon* gene for height introgressed, while the shorter plants most likely have disrupting introgression events in one or more of the Paragon height genes. Importantly, the wide spread of heights would make this new germplasm collection very useful for the study of plant height and possibly the discovery of novel height genes.



Σ 1022 lines

Figure 19: Height distribution of *T. monococcum* introgression lines during glasshouse grain multiplication. The (averaged) height for Paragon (the paternal donor during crossing) is indicated, showing the substantial number of lines with (significantly) higher and lower heights. The two extremely short (dwarf) and one extremely tall lines are indicated by green arrows.

Thousand grain weight (TGW) is a frequently used indicator for yield, provided there is also a large number of grains per ear. Paragon itself has a TGW of 36 ± 6 when grown in the field (data from Diversity Trial 2019 to 2022, provided by Andrew Riche) which correlates well with the extrapolated measurements from our glasshouse

multiplication (40 ± 3). We would have expected most introgressed lines to have a TGW (considerably) less than Paragon due to the much smaller grain size of *T. monococcum* and anticipated linkage-drag. However, **Figure 20a** shows that a considerable number of lines (35%) have an unanticipatedly higher TGW than Paragon.

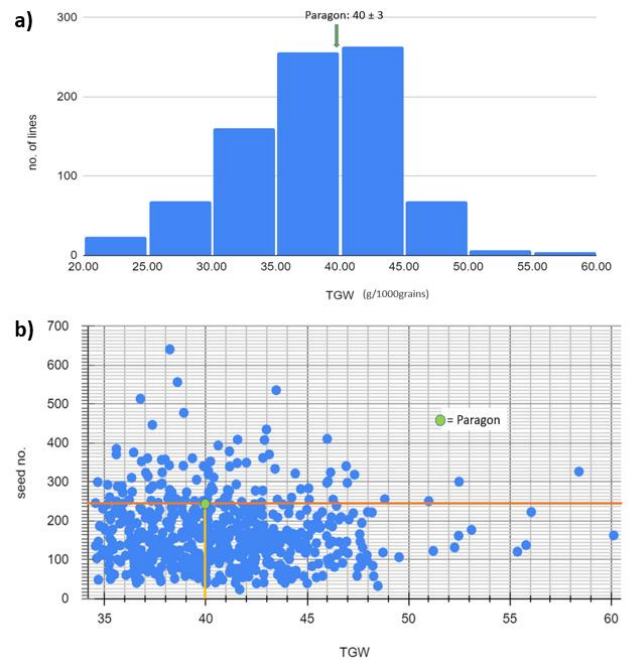


Figure 20: **(a)** distribution of TGW between introgressed lines, showing a surprisingly large number exceeding the TGW of Paragon and **(b)** scatterplot of seed number vs TGW for lines with a cut-off of $TGW \geq 35$. The position of Paragon is marked by a green circle. Any blue dot (introgressed line) to the right of the yellow line has a higher TGW than Paragon while all lines above the orange line have a higher grain number. The 42 lines both above and to the right indicate higher yield potential.

This was a very unexpected positive outcome. But TGW does not guarantee high yield - this requires a high grain number (grains/ ear) as well as high TGW. The scatterplot of grain number vs TGW for all lines (**Figure 20b**) revealed a total of 46 lines exceeding both the average TGW and grain number for Paragon (40 ± 3 and 252 ± 75). This is an extremely important result, because it suggests - and of course these values have to be corroborated in a field trial - that these lines do not necessarily need to be crossed to any high yielding varieties before progressing further - to the farmers' fields and beyond.

For **864 lines** there was enough grain obtained from the glasshouse multiplication (keeping at least 20 grains in reserve) to permit drilling in a small plot **Spring 2023 field trial** on March 2nd. Each line was drilled into a single 1m² plot, in 4 rows with a maximum of 100 grain. The trial was designed in 8 blocks of 6 x 16 lines and also includes Paragon control plots (1/block) (**Figure 21**).

The aims of the 2023 field trial are severalfold: 1) allow scoring for disease resistance, 2) phenotyping multiple plant, ear and root architecture traits, 3) generating enough grain to distribute to UK breeders, 4) engaging with the wider UK wheat research community to phenotype this trial for their specific traits, 5) invite farmers (NFU Hertfordshire already engaged) to visit the trial and discuss the multifactorial potential benefits. Towards making this wider interaction a success, we created a new network – **NIFTYR** (pronounced “niftier”...) **Network for Introgression Field Trial for Yield and Resistance**. This has been promoted to the UK wheat research community, as well as all WGIN stakeholders, and also at **Monogram 2023** in Reading. **To join** and subsequently be kept informed about the progress of the trial and when to best visit, please **scan the QR code** (to the left) and answer only six short questions, including contact details and a short outline of research interests and desired traits from this project. **NIFTYR membership is free and open to all.**



Figure 21: (a) layout of the *Triticum monococcum* Introgression spring field trial. Plot 1 is marked by the blue arrow while the orange arrow indicates the last plot, plot 864. The position of the blue arrow coincides with the position of the tractor in (b). The (barely distinguishable) colours of individual plots in (a) reflect the parental origin of the 8 F1Complex plants.

For further information on this WGIN project contact **Mike (Michael Hammond-Kosack)** at Rothamsted (wgin.defra@rothamsted.ac.uk).

Section 3 Previous events

The latest **WGIN Stakeholders' Meeting**, which was actually the 20th, took place on **February 6th, 2023**, as a hybrid event at the John Innes Centre. The meeting was recorded in its entirety and is available to watch or download in three parts from the WGIN website (www.wgin.org.uk). The panel discussion topic was **'The Role of Genetics in Integrated Pest Management'** and our panellists were **Jenna Watts** (AHDB), **Holly Alpren** (defra), **Nick Bird** (KWS), **Tom Allen-Stevens** (BOFIN) and **Phil Humphrey** (AICC, NIAB) and the discussion was chaired by **Peter Shewry** (RRes). Their contributions were greatly appreciated.

Many thanks to **Sarah Tolland** and her team at JIC for making the whole meeting run very smoothly as well as providing the recordings.

Section 4 Upcoming events

- The next **WGIN annual Stakeholders' Meeting** will take place on **Thursday February 8th, 2024**, again at the **John Innes Centre, Norwich**. This will be another hybrid meeting, but because Covid is now officially over, at least according to the WHO, with emphasis on in-person attendance. If you had any **suggestions for the Panel discussion** topic, please email Peter Shewry (peter.shewry@rothamsted.ac.uk) or Mike Hammond-Kosack (wgin.defra@rothamsted.ac.uk).
- The long overdue (thanks to Covid and the invasion of Ukraine) **Baltic Wheat workshop**, organised by Peter Shewry, is now taking place from **23rd to 25th of May in Malmö, Sweden** and involves 40 wheat breeders and researchers from the UK, Scandinavia and Baltic countries.

Section 5 News

- **Javier Palma-Guerrero** unfortunately had to leave Rothamsted for family reasons and has taken up a new role in EU project management in Spain.
- The **new leader of the Take-all team** at Rothamsted is **Dr. Nida Ghor**. She originates from Pakistan and has been working on wheat for the last 12 years. Nida obtained her PhD from Kansas State University majoring in Wheat Genetics on a Fulbright Scholarship.

- The WGIN Management would like to thank **David Feuerhelm** (Syngenta) for being a stalwart at Management meetings for over two decades. He has now retired, and we wish him all the best.
- The WGIN Management would also like to thank **Ruth Bryant** (RAGT) for her many meaningful contributions to WGIN for over a decade. She has now taken up a more senior position at **Syngenta** in Stein (Switzerland). We wish her success in her new role.

Section 6 Publications 2021 to 2023

- **Henry Tidd***, Jason J Rudd, Rumiana V. Ray, Ruth Bryant and Kostya Kanyuka (2023). A large bioassay identifies *Stb* resistance genes that provide broad resistance against *Septoria tritici* blotch disease in the UK. *Front. Plant Sci.*, 09 January 2023 Sec. Plant Pathogen Interactions Vol 13. <https://doi.org/10.3389/fpls.2022.1070986> (Part of the Research Topic Cereal Leaf Blights, Volume II). Note: all authors, except Ruth Bryant and Kostya Kanyuka, are not part of the WGIN project.
- **Maria Oszvald***, Kirsty L Hassall, David J Hughes, Adriana M Torres-Ballesteros, Ian Michael Clark, Andrew Bernard Riche, Sigrid Heuer (2022). Genetic diversity in nitrogen fertilizer responses and N gas emission in modern wheat. 2022. *Frontiers in Plant Science*, section Plant Nutrition. <https://doi.org/10.3389/fpls.2022.816475> Note: all authors, except Andrew Riche, are not part of the WGIN project.
- **Palma-Guerrero J.**, Chancellor, T., Spong, J., Canning, G., Hammond, J., McMillan V. E. and Hammond-Kosack, K.E. (2021) Take-all disease: New insights into an important wheat root pathogen. *Trends in Plant Sciences* 26, 836-848. (RRes press release - 21st March 2021) <https://www.rothamsted.ac.uk/news/taking-take-all>
- **Simons***, A. L., Caulfield, J.C., Hammond-Kosack, K. E. , Field, L.M. and Aradottir, G. I. (2021) Identifying aphid resistance in the ancestral wheat *Triticum monococcum* under field conditions. *Nature Scientific Reports* Article No 13495.

- **Hammond-Kosack, M.C.U., King, R., Kanyuka, K. and Hammond-Kosack, K.E. (2021)** Exploring the diversity of promoter and 5'UTR sequences in ancestral, historic and modern wheat. *Plant Biotechnology Journal* 19, 2469-2487 + front cover (RRes press release - 28th October 2021 - <https://www.rothamsted.ac.uk/news/new-targets-crop-genetic-improvement-found>)
- **Alba Farre Martinez*** and Clare Lister, Sue Freeman, Jun Ma, Simon Berry, Luzie Wingen, Simon Griffiths (2021) Resolving a QTL complex for height, heading, and grain yield on chromosome 3A in bread wheat. *Journal of Experimental Botany* (<http://dx.doi.org/10.1093/jxb/erab058>)
- **Elizabeth A. Chapman***, Simon Orford, Jacob Lage, Simon Griffiths (2021) Capturing and Selecting Senescence Variation in Wheat. *Frontiers in Plant Science* (<http://dx.doi.org/10.3389/fpls.2021.638738>)
- **Chapman***, E. A., Orford, S., Lage, J., & Griffiths, S. (2021). Delaying or delivering: identification of novel NAM-1 alleles that delay senescence to extend wheat grain fill duration. *Journal of Experimental Botany*, 72(22), 7710-7728.
- **Dreisigacker***, S., Burgueño, J., Pacheco, A., Molero, G., Sukumaran, S., Rivera-Amado, C. & Griffiths, S. (2021). Effect of flowering time-related genes on biomass, harvest index, and grain yield in CIMMYT elite spring bread wheat. *Biology*, 10(9), 855.
- **Zhou, H., Riche, A. B., Hawkesford, M. J., Whalley, W. R., Atkinson, B. S., Sturrock, C. J. and Mooney, S. J. (2021)** Determination of wheat spike and spikelet architecture and grain traits using X-ray Computed Tomography imaging. 2021. *Plant Methods*. 17 (article), p. 26. <https://doi.org/10.1186/s13007-021-00726-5>

*non-WGIN funded 1st authors

For further information on any aspect of the WGIN project please go to www.wgin.org.uk, contact us at wgin.defra@rothamsted.ac.uk or message us on Twitter (@WheatGIN). Contributors to this newsletter were: **Kim Hammond-Kosack**(RRes), **Clare Lister**(JIC), **Andrew Riche**(RRes), **Javier Palma-Guerrero**(formerly RRes), **Lawrence Bramham**(RRes) and **Mike Hammond-Kosack**(RRes, editor).

